THE AVERSIVE STIMULUS PROPERTIES OF SCOPOLAMINE: AN INVESTIGATION USING THE RESPONDENT CONDITIONING PARADIGM

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ABSTRACT

The objective of the experiments in this study was to investigate the proposed aversive stimulus properties of scopolamine hydrobromide (1.2 mg/kg I.P.) All research designs were based on the respondent conditioning paradigm. In Experiment I, scopolamine was used as a (putative) aversive UCS in order to produce conditioned suppression of licking to a light CS. No evidence of suppression was found. It was proposed that this may have been due to the inability of the UCS itself to suppress responding. In a second experiment, scopolamine injections were paired with one side of a shuttlebox, and saline injections were paired with the other side. Subsequent free choice tests yielded a constant preference for the saline associated side. In a further test where subjects were injected 20 minutes prior to confinement, no avoidance of the scopolamine associated side was observed. These results suggest that the onset of scopolamine effect is aversive.

CHAPTER I

INTRODUCTION

Since ancient times man has recognised the existence of drugs known to alter his state of consciousness. Such drugs have been used variously to restore mental health, explore the mind and induce euphoria. It was said that the Homeric physician Polydama presented Menelos and Helen with "a drug against sorrow and anger, a drug to survive despair" on their way home to Troy.

Today such substances are known as psychotropic drugs. Most psychotropic drugs were discovered with little premeditation. The drugs were simply known to "work" often with little or no knowledge of their modes of action. In the past decade the sciences of neuropharmacology and psychopharmacology have developed and grown rapidly in an attempt to fill this vacuum. Neuropharmacology is concerned with the study of the effects of drugs on nervous tissue; psychopharmacology is concerned with the study of the effects of drugs on behaviour. Advances in the understanding of the effects of psychotropic drugs have been so prodigious that a new research paradigm has evolved. Instead of being concerned solely with the understanding of the mechanisms of drug action, many

researchers now employ psychotropic drugs as tools to elucidate brain function. In such a pursuit it has come to be recognised that the information processing activities of the brain are reliant upon the unique ability of neurons to communicate with one another, through the chemical mediation of neurotransmitters released at synapses. It has been proposed by various investigators that many psychotropic drugs may act primarily to alter some facet of synaptic transmission. Enhancing or retarding the probability of activation of the post synaptic neuron.

Acetylcholine

The first substance to be established as a neurotransmitter was the amine, acetylcholine. In 1926, after 5 years' research, Otto Loewi proposed that the stimulation of the vagus nerve and the consequent reduction in rate and strength of cardiac contraction in an isolated frog's heart was under the control of acetylcholine. It was established that acetylcholine was liberated by vagus stimulation and the evidence suggested that the ester itself was responsible for transmitting the effects of nerve stimulation to the heart. Today it is thought that acetylcholine is a transmitter substance in various parts of the nervous system, namely (a) all post ganglionic fibres of the parasympathetic division of the autonomic nervous system and in a few post ganglionic fibres of

the sympathetic division; (b) the preganglionic fibres of the parasympathetic and sympathetic divisions; (c) the motor nerves at the skeletal muscles. Recently the function of acetylcholine in the central nervous system has been recognised which has given rise to many hypotheses regarding the connection between acetylcholine and behaviour. High concentrations of acetylcholine are found in particular in the cortex, nucleus caudatus, hypothalamus, thalamus, midbrain and corpus callosum.

Anticholinergics

Attempts to identify behaviour that may be influenced by central acetylcholine activity have often involved the use of anticholinergic drugs. Such drugs have their pharmacological basis in the antagonism of the muscarinic actions of acetylcholine. Muscarinic synapses are those which can be stimulated by muscarine administration. All the actions of muscarine can be reproduced by acetylcholine although acetylcholine has other effects not shown by muscarine. Although muscarine does not occur in animal tissues it is convenient to speak of the muscarinic actions of acetylcholine as a separate pharmacological entity. Experimental evidence suggests that a large proportion of central acetylcholine receptors are muscarinic in type. It has been established that the anticholinergic antagonism of the muscarinic actions of acetylcholine is of a competitive nature.

It competes with the neurotransmitter for the acetylcholine receptors on the post synaptic membrane.

In psychopharmacological research the most commonly used anticholinergics are atropine and scopolamine (hyoscine). Traditionally explanation of the behavioural changes associated with the administration of these drugs has been in terms of antagonism of a central cholinergic system. However in recent years the validity of postulating such unitary central mechanisms for the control of complex behavioural patterns has been questioned. It has been suggested that simpler and more parsimonious explanations may have been overlooked. The stimulus properties of anticholinergics is one such explanation which has often been neglected.

Among the various behavioural changes associated with the administration of anticholinergics, is the effect of the drugs on preferences for novelty. It is proposed that this effect, previously explained solely in terms of antagonism of the central cholinergic system, may well be influenced by the stimulus properties of the drug.

CHAPTER II

LITERATURE REVIEW

A. SCOPOLAMINE AND HABITUATION TO NOVELTY

The suggestion that habituation to novelty is under the control of central acetylcholine neural pathways was initially propsed by Carlton (1968, 1969). He proposed that habituation was a process which involved cholinergic inhibition of responses to stimuli which do not act as rewards in the conventional sense. The disruption of this inhibitory influence by the action of anticholinergics (commonly scopolamine) should impair the process of habituation leading to a more prolonged responsiveness to novelty. As Hughes (1978) notes, although some studies of the effect of scopolamine on exploratory motivated behaviour have supported Carlton's hypothesis, much of experimental evidence has been difficult to reconcile with such views. Examples include the ineffectiveness of anticholinergics in habituation of the "headpoke" exploratory response (Walters and Block, 1969; Feigley and Hamilton, 1971), ambulatory activity (Walters and Block, 1969; Green, Joyce and Summerfield, 1975), change of active choices in a novel T-maze arm (Leaton and Buck, 1968) and distraction from

drinking by a novel tone (File, 1976).

Two basic research strategies have been employed in assessments of anticholinergic drug effects on habituation to novelty. Interest in the role of the stimulus properties of anticholinergics has arisen from both experimental paradigms.

(i) Habituation both develops and is assessed while the subject is drugged (Paradigm I, Carlton 1968).

(a) Spontaneous Alternation

It has been observed that the administration of scopolamine typically disrupts spontaneous alternation. Spontaneous alternation is the tendency for animals to alternate successive unrewarded entries of the arms of a T or Y maze. This is generally acknowledged as a tendency to choose the more novel option, i.e. the arm of the maze less recently entered. Alternation can therefore be seen to reflect habituation to the novelty of stimuli encountered during the preceding choice. Anticholinergics, said to attenuate habituation should therefore cause a reduction in alternation. Several authors have demonstrated this (Parkes, 1965; Douglas and Isaacson, 1966; Squire, 1969; Drew et al, 1973; Kokkinidis and Anisman, 1976). Maximum attenuation of habituation would be characterised by chance probability responding to the maze arms, indicating that both appear

equally novel to the subject. However it has been demonstrated by several authors that scopolamine treatment induces perseveration i.e. choosing the same arm as previously entered (Meyers and Domino, 1964; Hughes and Daly, 1977; Drew, Miller and Baugh, 1973; Kokkinidis and Anisman, 1976). As Hughes (1978) notes, if there is a reciprocal relationship between cholinergic inhibitory and adrenergic excitatory systems (Carlton, 1963), scopolamine and other anticholinergics could be expected to produce similar effects to adrenergic stimulants such as amphetamine. Amphetamine induced perseveration has been demonstrated (Kokkinidis and Anisman, 1976; Adkins, Packwood and Marshall, 1969). The implication of this drug in the production of spatial preferences (Glick, 1973) might indicate that perseveration with both scopolamine and amphetamine arises from repeated turning responses rather than from a tendency to select the same stimulus elements on successive opportunities.

(b) Scopolamine and Novelty Preference in Exploration Boxes

Other evidence also exists showing scopolamine to produce preferences for familiar stimuli. When rats are confined to one half of an exploration box and then later given access to both the same and the previously inaccessible side they tend to prefer the latter, more novel

alternative (Hughes, 1968). Hughes et al (1975, 1977b) demonstrated that if scopolamine is administered between confinement and testing the reverse situation prevails. They tend to prefer the previously experienced familiar half. Such a preference cannot be explained by a simple response bias as is possible with scopolamine induced perseveration. The behaviour was undoubtedly a genuine stimulus preference characterised by novelty avoidance. An impaired habituation hypothesis cannot account for this preference either because by showing a preference for familiarity the rats were efficiently discriminating between the novelty-familiarity stimulus characteristics of the two halves.

Berger (1972) demonstrated that scopolamine was an effective CS in the conditioning of taste aversions to milk drinking. He found that taste aversions were produced only if the drug was administered immediately after or for four hours after drinking. Aversions were produced after a single session. Aitken (1972) demonstrated that rats show preferences for familiar rather than novel stimuli in the presence of stressful experiences. In his experiment, rats were confined to the stem of a T maze in which glass doors prevented access to black-white choice arms. On a subsequent free choice trial when both arms were either black of white, rats shocked prior to choice spent a greater amount of time in the unchanged arm, whereas unshocked controls preferred the

changed arm. Thus the previously shocked rats appeared to be demonstrating a preference for the more familiar of the two arms. O'Connell (1974) demonstrated that rats which had been deprived handling during infancy spontaneously alternated less than handled animals. It was indicated that the test procedure was probably more stressful for the non handled animals than it was for the handled animals. These factors led to the suggestion by Hughes (1975) that the aversive stimulus properties of scopolamine might be responsible for the increasing preference for familiar stimuli.

(ii) Habituation develops in a drugged state but is assessed later without drug (Paradigm II, Carlton 1968).

Several studies have employed an experimental design based on this paradigm, involving the confinement of scopolamine and saline treated rats to an experimental chamber and then some days later, water depriving them and returning them to the chamber which has since been provided with a water dispenser. The animals who have been previously exposed to the chamber after scopolamine administration tend to take longer to approach the water tube than the saline controls (Carlton and Vogel, 1965; Anisman et al, 1976). It has been presumed that these increased drinking latencies were the result of increased exploratory behaviour due to scopolamine-induced habituation

deficits in the initial session. Such an index of exploratory behaviour did not seem satisfactory, thus Hughes and MacMahon (1976) devised a situation whereby habituation deficits were determined by confining scopolamine and saline treated rats to one half of an exploration box and then, one day later, allowing them free access to the same and previously inaccessible novel half without drug administration. Previously drugged animals showed significantly lower tendencies to visit the novel half to the point where the familiar half was preferred. As was the case with the first experimental paradigm, maximum attenuation of habituation should have appeared as no significant preference for either side. This result has since been replicated and been shown to be due to neither state change nor state-dependent effects (Hughes and Daley, 1977b). It was suggested by the authors (Hughes and MacMahon, 1976) that during prior confinement, the scopolamine treated animals may have developed a conditioned aversion to some part of the experimental procedure. Thus when later tested without drug, the conditioned aversive stimuli may have led the rats to avoid novel stimuli in the manner shown for other aversive experiences (Aitken, 1972). A similar explanation might also account for the larger drinking latencies in previously scopolamine treated animals (Carlton and Vogel, 1965; Anisman et al, 1976).

Various studies have demonstrated anticholinergics

to be discriminable and conditionable interoceptive stimuli. Overton (1969, 1971) has demonstrated that rats can easily discriminate a wide range of dosages of scopolamine and other anticholinergics from non drug conditions, in a T maze shock escape situation. Kubena and Barry (1969) demonstrate that this discrimination response to scopolamine will generalise to atropine, suggesting a similarity in perceived drug states. Koral et al (1966) and Lang et al (1966) have demonstrated that responses of paradoxical salivation and classical mydriasis can be respondently conditioned using atropine. As previously mentioned, it has also been reported that scopolamine is an effective stimulus in the conditioning of taste aversions (Berger, 1972).

Quaternary Anticholinergics

Methylation of the nitrogen atoms of atropine and scopolamine produce their respective quaternary derivatives, namely scopolamine methyl nitrate and atropine methyl nitrate. These drugs, while continuing to produce peripheral anticholinergic effects, do not pass the blood brain barrier as readily as their non methylated counterparts and thus have very little effect in the central nervous system (Goodman and Gilman, 1965). Comparison of the effects of methylated and non methylated anticholinergics has traditionally been used to assess the relative contributions of cholinergic activity in

the central nervous system and the peripheral nervous system in influencing behaviour.

While most authors have failed to observe any behavioural change with methyl scopolamine, it has been demonstrated that quaternary anticholinergics are in fact capable of influencing reasonably complex behaviour. Leaton (1969) has demonstrated that in a brightness discrimination task reinforced by an opportunity for exploration, rats injected with methyl scopolamine made significantly more exploratory choices than saline controls. Green, Joyce and Summerfield (1975) in a study of the habituation of exploratory behaviour in rats found that the related quaternary anticholinergic scopolamine butylbromide to decrease initial within session activity in a novel Y maze. Hicks (1976) demonstrated that injection of methyl scopolamine served to facilitate the habituation of susceptibility to tonic immobility in chickens.

Koral et al (1966) demonstrated that responses of paradoxical salivation and classical mydriasis could be respondently conditioned using atropine methyl nitrate and suggested that the conditioning of such responses was the result of peripheral anticholinergic drug action. Berger (1972) also reports the peripherally acting quaternary anticholinergic methyl scopolamine to be more effective than scopolamine itself in establishing conditioned aversions. He proposes therefore that it is likely that these anticholinergics cause food aversions by their peri-

pheral action. Horsburgh (1978) using the same experimental design as Hughes and MacMahon (1976) has demonstrated a preference for familiarity using methyl scopolamine. Such evidence could lead to the suggestion that novelty avoidance is possibly due to scopolamine's peripheral effects. However there is also evidence that quaternary anticholinergics are ineffective in influencing other exploratory indexes such as spontaneous motor activity (Meyers and Wilchin, 1969) and the "head poke" test (Williams, Hamilton and Carlton, 1974). However as Cappell and Le Blanc (1973) have demonstrated, whether a drug's effects are reinforcing or aversive may depend upon the particular type of behaviour under consideration.

The Aversive Novel Experience Hypothesis

The aversive stimulus hypothesis need not however depend solely on the peripheral properties of anticholinergics (Hughes, 1978). The central action of a drug might in itself be aversive, even if it only constitutes a highly novel experience. Amit and Baum (1970) proposed this after finding that both alcohol and hashish administration increased the resistance to extinction of an avoidance response. Since these drugs are pharmacologically unrelated and the response was obtained in both mice and rats, it was concluded that it was unlikely that the response was a function of the pharmacological properties of a specific drug. It was suggested that the experience

of being in a drugged state for a pharmacologically naive subject is aversive and thus acts to increase fear in the avoidance situation. This is of particular relevance as most studies of the effect of scopolamine on novelty choices have involved subjects who were pharmacologically naive prior to drug treatment.

B. TESTING THE AVERSIVE STIMULUS PROPERTIES OF DRUGS

The question of how one ascertains whether or not an animal finds a drug effect aversive is not one that is easily answered. There are at least three experimental paradigms (of varying validity) available for the demonstration of such an effect. Each of these paradigms involves a conditioning procedure in which the drug is employed either as an unconditioned stimulus (respondent) or as a punisher (instrumental). In using these paradigms it is important to avoid confounding due to any direct effects of the drug on performance of the behaviour for which the dependent variables are measured, or on any underlying associative processes. The paradigms to be discussed are:

(a) Conditioned suppression

(b) Respondent conditioning of taste aversions and choice behaviour

(c) Instrumental conditioning of taste aversions and choice behaviour.

It should be noted that these paradigms are not necessarily mutually exclusive (or exhaustive). Experimental designs may often encompass more than one of the paradigms.

(i) Conditioned Suppression

This procedure was derived from the work of Estes and Skinner (1941) who reported the production of the conditioned emotional response. An originally neutral stimulus is paired with an unconditioned stimulus in a respondent conditioning paradigm (i.e. non contingent on the animal's behaviour), and the measure of conditioning is whether the disruption of some ongoing appetitive behaviour (such as bar pressing) which originally occurred to the unconditioned stimulus begins to occur to the originally neutral stimulus. The paradigm has traditionally involved the use of shock as the unconditioned stimulus and the production of the conditioned suppression was seen as being due to the association of the CS with the aversive properties of the UCS. It has been demonstrated that large numbers of drug types can successfully function as unconditioned stimuli (Cameron and Appel, 1972; Goldberg and Schuster, 1967; Whitney and Trost, 1970). It has also been demonstrated that many of these drug states can also function as reinforcers (Cappell and Le Blanc, 1973; Pickens and Harris, 1968; Wise, Yokel and de Wit, 1976). Cappell and Le Blanc (1973) have demonstrated aversive taste conditioning using amphetamine

within the range of doses which Pickens and Harris (1968) found rats would self inject. It would seem that there may well be considerable differences in the stimulus properties of drugs depending on whether they are administered contingent upon the subject's behaviour or non contingently. It may be the case that drugs which act as reinforcers in an instrumental conditioning paradigm may also act as aversive stimuli in a respondent conditioning paradigm. However it may also be the case that drugs which do not possess aversive stimulus properties are capable of producing conditioned suppression (Cameron and Appell, 1972). These authors suggest that it may be possible to distinguish between aversive and non aversive drug states on the basis of rate of extinction of the depression of behaviour. They suggest that extinction may be faster if the UCS drug state is non aversive than if it is aversive.

(ii) Respondent Conditioning of Taste Aversion and Choice Behaviour

Respondent conditioning has been used extensively in the demonstration of conditioned taste aversions. Rats and other animals have been shown to reject foods that are paired with X-rays, lithium or apomorphine (Garcia and Ervin, 1968; Revusky and Bedarf, 1967; Rozin, 1969). Berger (1972) has demonstrated that conditioned food aversions can be produced by moderate doses of drugs

which are widely used as therapeutic agents (scopolamine, amphetamine, chlorpromazine and benzodiazepine tranquillisers). The conditioning of taste aversions can also be seen as an example of conditioned suppression in that it essentially involves suppression of an ongoing response. The major difference between the majority of conditioned taste aversion studies and conditioned suppression studies is that the former often employs a single session conditioning paradigm whereas several sessions are employed in the latter. In this respect Amit and Baum's(1970) suggestion of the aversive nature of a novel drug state may be relevant in taste aversion studies. However where several sessions are employed, habituation to the drug state is likely to decrease any aversive effects associated with the novel properties of the drug state. Any reported effects are more likely to be due to more specific stimulus properties of the drug.

Reicher and Holman (1977) in further investigation of the effects of amphetamine as both a reinforcing and an aversive stimulus, devised an experiment whereby injections of amphetamine were associated with a compound stimulus consisting of a distinctive flavour and a distinctive location. Thus conditioned location preference and conditioned taste aversion could be established simultaneously. Their results demonstrated that amphetamine simultaneously produced a positively reinforced preference for the side associated with amphetamine, and

an aversion to the flavoured solution associated with the drug. This supported the suggestion by Cappell and Le Blanc (1973) that whether or not a drug state is aversive can depend upon the particular response under consideration.

It may also be the case that the nature of the CS is the dependent variable. In Reicher and Holman's study the effects of amphetamine were reinforcing when associated with exteroceptive stimuli (i.e. location) and aversive when associated with interoceptive stimuli (i.e. taste). The experiments in the present study involved the conditioning of exteroceptive stimuli.

Reicher and Holman (1977) demonstrated that conditioned taste aversions and location preferences could be produced, both by drug administration immediately prior to conditioning and drug administration 20 minutes prior to conditioning. Berger (1972) reported conditioning of taste aversions only if scopolamine was administered within four hours after access to the milk solution to which the drug effect was to be conditioned. Drug injection 30 minutes before drinking had no effect. This negative effect was explained in terms of Pavlovian conditioning procedures requiring that the conditioned stimulus (i.e. milk) precede the UCS (i.e. drug). However the results of Reicher and Holman's delayed injection study place such an explanation in some jeopardy. One possible alternative explanation for

Berger's result which he in fact mentions but attributes no importance to is that "... the rats that had been 'poisoned' with the drug (i.e. given the drug prior to conditioning) usually licked a few times before withdrawing to the back of the cage, and frequently assuming a crouching and freezing posture" (Berger, 1972, p. 24). This observation is consistent with various reports that the administration of scopolamine in similar doses decreases liquid consumption (Stein, 1963; Prabham and Roth, 1968). This effect was also produced in one of the experiments in this study (Chapter 4). These observations lead to the suggestion that the subjects may not have had sufficient exposure to the CS to produce a conditioned aversion.

(iii) Instrumental Conditioning of Taste Aversions and Choice Behaviour

Berger's production of taste aversions with drug administration following the exposure to the milk can also be explained in terms of the third experimental paradigm - that involving instrumental rather than respondent conditioning. It could well be the case in this situation that the administration of scopolamine after drinking was in fact a punishing event. A punishing stimulus is defined as one which "can suppress behaviour which precedes its presentation" (Kelleher and Morse, 1964). It has been demonstrated that the contin-

gent administration of drugs such as amphetamine and chlorpromazine as punishing stimuli are effective in the conditioning of taste aversions to saccharin (Rozin and Kalat, 1971; Cappell and Le Blanc, 1973; Cappell, Le Blanc and Herling, 1975).

Similarly, the design could also be used to assess instrumental conditioning of location preferences. Such a design could involve a T maze shock escape task similar to that employed by Overton (1969) in his work on drug state discrimination. The runway of the T maze would be electrified and both goal boxes shock free. Initial trials may need to involve forced turns into both goal boxes to ensure that the subject experiences both as being shock free. Turning into one goal box would always result in saline injection whereas turns into the other goal box would result in injection of the particular drug under consideration. Any ensuing preference for or avoidance of the goal box leading to drug injection could be explained in terms of the reinforcing or aversive stimulus properties, respectively, of the drug. The shock escape task could be exchanged for a design involving water reinforcement in the goal boxes. The effectiveness of such a design would be dependent upon relative reinforcing properties of the water and the aversiveness of the injection procedure. If the latter outweighs the former, it is likely that the animals would enter neither goal box.

The experiments in the present study have involved

the first two paradigms proposed for the investigation of the aversive stimulus properties of drug states. Preliminary studies of the stimulus properties of scopolamine have been carried out using the conditioned suppression paradigm and the respondent conditioning location preference paradigm. Drug administration in both these paradigms is not contingent upon any particular response by the subject. It was felt that these techniques were more appropriate to the investigation of the role of the stimulus properties of scopolamine in habituation to novelty studies, where the drug has also been administered non contingently.

CHAPTER III

GENERAL METHODS

A. SUBJECTS

The subjects used in all experiments were naive New Zealand random breed hooded male rats from the Department of Psychology, University of Canterbury, breeding colony. The subjects housed four to a cage, were reared in single sex groups. The colony room maintained constant temperature and humidity, and a 12 hours light/12 hours dark light cycle was in operation. All testing was conducted in the dark phase, between 6.30 a.m. and 6.30 p.m.

B. APPARATUS

In all experiments, background noise was generated by a Lafayette White Noise Generator, Model 15011. All decibel (dB) recordings were taken with a Dawe Instruments Ltd. Transistor Sound Level Meter, Model 1400E (A scale). The microphone was placed in the apparatus in the approximate position in which the subject would have been. Illumination levels were recorded with a Toshiba Photocell Illuminometer, Model SPI-5. The detector was placed centrally on the floor of the apparatus and pointed

directly towards the light source.

C. DRUGS

The drug used in all experiments was scopolamine hydrobromide (Hyoscine hydrobromide injection; 0.6 mg/ml ampules; McGaw Ethicals). The dose used was 1.2 mg per kg body weight. This dosage is similar to dosages used in the majority of studies reviewed. Control injections were of sterile isotonic saline solution (9 gms analar grade NaCl per litre distilled water). Injection volume in all instances was 2 ml fluid per kg body weight. All injections were given intraperitoneally.

D. EXPERIMENTAL SESSIONS

All experimental sessions were of 20 minutes duration. Subjects received no more than one session per day.

CHAPTER IV

EXPERIMENT I

A. INTRODUCTION

The objective of this first experiment was to investigate the aversive stimulus properties of scopolamine using the conditioned suppression paradigm. The chosen dependent response was licking at a water tube. Scopolamine administration (UCS) was paired with a flashing light (CS). The CS was subsequently tested for its ability to suppress responding.

B. METHOD

(i) Subjects

Eight subjects were used. Their age range at time of testing was 100 - 118 days (mean = 104 days) and their weight range was 250 - 309 grams (mean = 285 grams). All subjects were provided with ad libitum food in the home cage. During testing and for 14 days prior to testing, the animals were maintained on a 1 hour on/23 hours off water deprivation schedule. During testing the animals were given access to water for 20 minutes whilst in the experimental apparatus and were given the remain-

ing 40 minutes access in their home cages after completion of the test session. In the 14 days prior to testing and during the conditioning sessions, access to water was given entirely in the home cage.

(ii) APPARATUS

The rats were individually tested in an experimental chamber measuring 24 x 22 x 18 cm (L x W x H). The sides, back and top of the chmber were constructed of white painted aluminium. The floor of the chamber was composed of .30 cm brass rods spaced 1.2 cm apart. The front of the chamber was constructed of transparent perspex. On one side of the chamber there was a 4.5 cm diameter hole over which a perspex plate was mounted. Through the 2.5 cm diameter hole in the perspex plate a stainless steel drinking nipple could be inserted and retracted. Rats could obtain water by pressing a small pin in the centre of the nipple. The nipple was connected via a plastic pipe to a graduated burette mounted outside the apparatus. A Grason-Stadler Drinkometer Sensing Head, Model E4690-A, was connected to the drinking nipple and the grid floor of the chamber. On the ceiling of the chamber directly above the drinking aperture was a 5 cm diameter plate of translucent perspex above which was mounted the light source. Resting illumination in the chamber was 41 lux. The houselights could be flashed on

and off with a peak illumination level of 120 lux. The flash occurred once per second for a duration of 50 msecs.

The experimental chamber and drinkometer sensing apparatus were enclosed within a ventilated lightproof enclosure. A small magnifying lens in the door allowed observation of the subject. Background noise inside the experimental chamber with the ventilator fan operating was 67 dB. Additional white noise from the white noise generator was provided via a speaker situated in the ceiling of the lightproof enclosure. Total masking noise in the experimental chamber was 80 dB.

Onset and offset of the flashing light CS was automatically controlled by Pye Hi-Log programming equipment. An Esterline-Angus operations recorder was used to record responses (i.e. each lick) and stimulus events. Counters also recorded number of responses emitted during the pre-CS period, the CS period and the total number of responses emitted over the whole session. Water consumption over the session was recorded from the graduated burette.

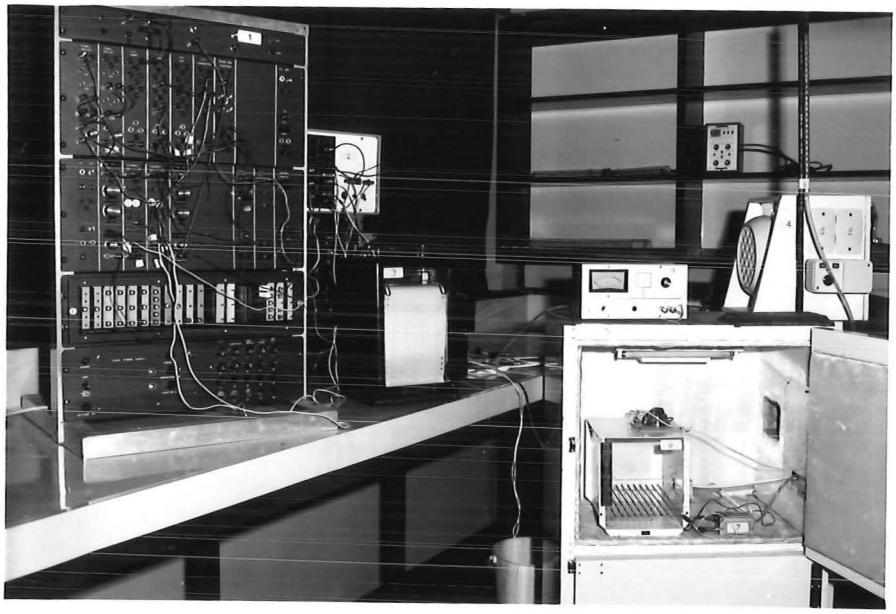

FIGURE 1: The experimental apparatus

1. Pye Hi-Log programming equipment
2. Response counters
3. Esterline Angus Operations Recorder
4. Ventilator fan
5. Lightproof enclosure
6. Experimental chamber
7. Grason-Stadler Drinkometer Sensing Head (Model E4690-A)
8. Graduated burette
9. Lafayette White Noise Generator (Model 15011)

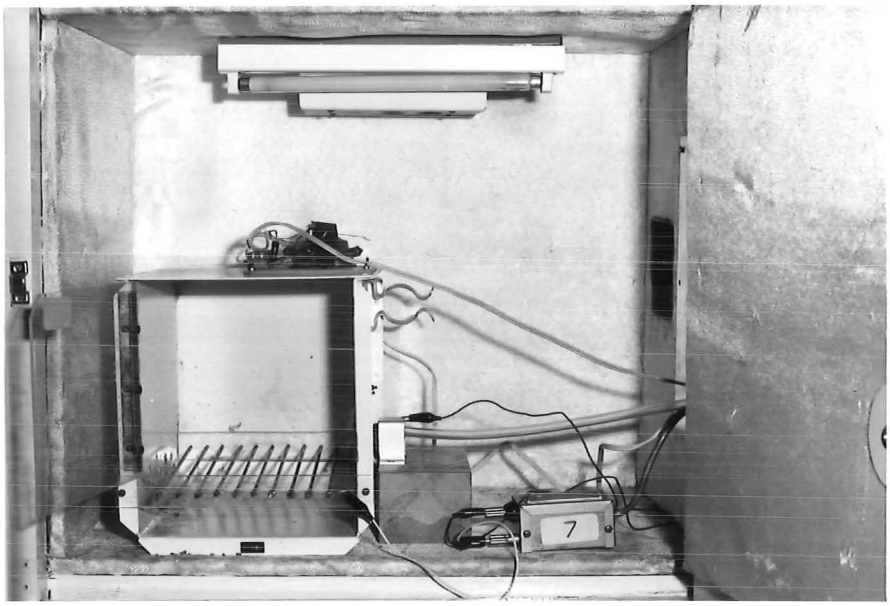

FIGURE 2: The experimental chamber

(iii) Procedure

There were twenty consecutive daily sessions.

(a) Adaptation

On days 1 - 8 each subject received one session per day in the experimental chamber, without injection. On day 1 the drinking nipple was inserted 2 cm into the experimental chamber. On day 2 and in all sessions thereafter, the nipple was retracted to 2 mm outside the experimental chamber. On days 1 and 2 the total number of responses and the total amount of water consumed over each session was recorded. On days 3 - 8 each session was divided into 4 x 5 minute intervals. The first minute of each interval was designated the pre CS minute. Number of responses made in the pre CS minutes was recorded after each session. The second minute of each interval was designated the CS minute. During this minute, the CS (the flashing of the houselight) was presented. The number of responses made in the CS minutes was recorded after each session. The total number of responses and the total amount of water consumed over the whole session was also recorded.

(b) Conditioning

On days 9 - 14 each subject was given an injection of either saline or scopolamine twenty minutes before

being placed in the experimental chamber. Each subject was given scopolamine or saline on alternate days. Subjects were randomly assigned to two groups so that on one day, one group received scopolamine and the other saline, and on the following day vice versa. All subjects received three scopolamine sessions (60 minutes total) and three saline sessions (60 minutes total) during the conditioning phase of the experiment. During those sessions in which scopolamine was administered, the CS was presented throughout the session. During this phase of the experiment, the drinking nipple was removed from the experimental apparatus.

(c) Post Test for Conditioned Effects

On days 15 and 16 the procedure followed on days 3 - 8 was duplicated. The drinking nipple was returned to the experimental chamber. No injections were given.

(d) Post Test for Unconditioned Effects

On days 17 - 20 the subjects again received an injection of either scopolamine or saline twenty minutes before being placed in the experimental chamber. The subjects were again divided into two groups and received scopolamine or saline on alternate days. During these sessions the subjects had access to water. The CS was not presented during these sessions. Total number of

responses and amount of water consumed was recorded.

C. RESULTS

The number of responses (licks) made in the CS and pre CS minutes of each session on days 3 - 8 (before conditioning) and on days 15 and 16 (after conditioning) were used to produce a single conditioned suppression ratio for each animal on each day. The conditioned ratio was calculated by:

$$\frac{R_{CS}}{R_{CS} + R_{PRE\ CS}}$$

R_{CS} = number of responses in CS period

$R_{PRE\ CS}$ = number of responses in pre CS period

The greater the conditioned suppression of responding the closer the ratio will be to zero. A ratio of 0.5 would mean the subject was responding equally in both the CS and pre CS periods, i.e. no suppression.

Figure 3 illustrates the mean daily conditioned suppression ratios for the group before and after the conditioning sessions. On the six days prior to conditioning sessions, the mean daily conditioned suppression ratios were 0.43, 0.48, 0.43, 0.55, 0.42 and 0.48

consecutively. These ratios tended to be less than .50 due to the temporal order of the pre CS and CS periods. As the pre CS period occurred before the CS period in each five minute interval, the subjects tended to make more responses in the former as overall more responses were made in the first part of the session than in the second.

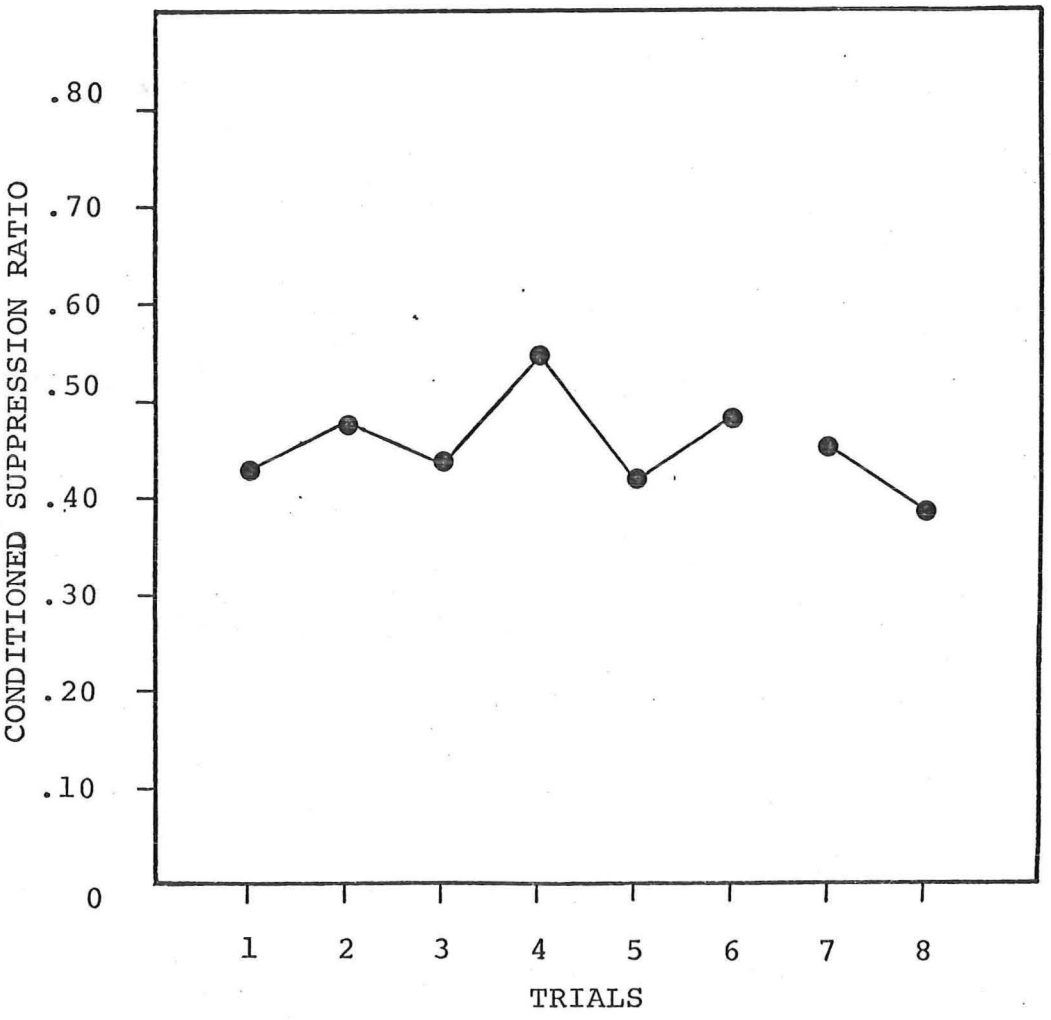

FIGURE 3: Conditioned suppression ratios before (trials 1-6) and after (trials 7-8) conditioning sessions.

On the two days after conditioning the mean daily conditioned suppression ratios were 0.46 and 0.39, respectively. One way ANOVA comparing ratios before and after conditioning showed no significant differences ($F(7,49) = 0.9\text{-}3$ N.S.) indicating that the subjects were maintaining approximately the same ratios of responding both prior to and after the conditioning sessions.

Figure 4 displays mean daily water consumption for the group before and after conditioning sessions. The subjects' increasing adaptation to the box and mastery of the drinking task is shown by the consistent increase in amount consumed from days 1 - 5. Comparison of pre and post conditioning water consumption shows no significant difference ($F(1,49) = 0.24$ N.S.).

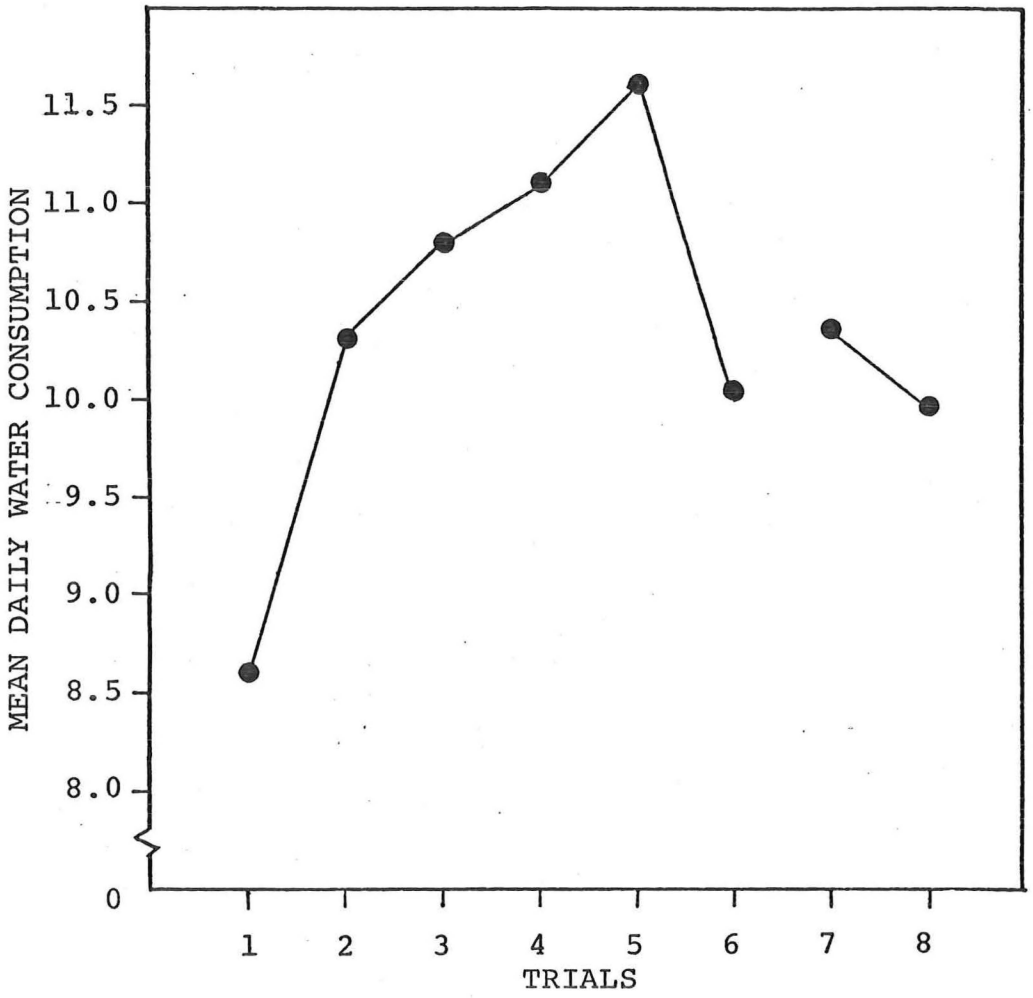

FIGURE 4: Mean daily water consumption (ml) before (trials 1-6) and after (trials 7-8) conditioning sessions.

Figure 5 illustrates total session responding means for the group before and after conditioning. Although there is a visible trend towards a decrease in responding in the post test, this is not significant ($F(7,49) = 1.17$ N.S.)

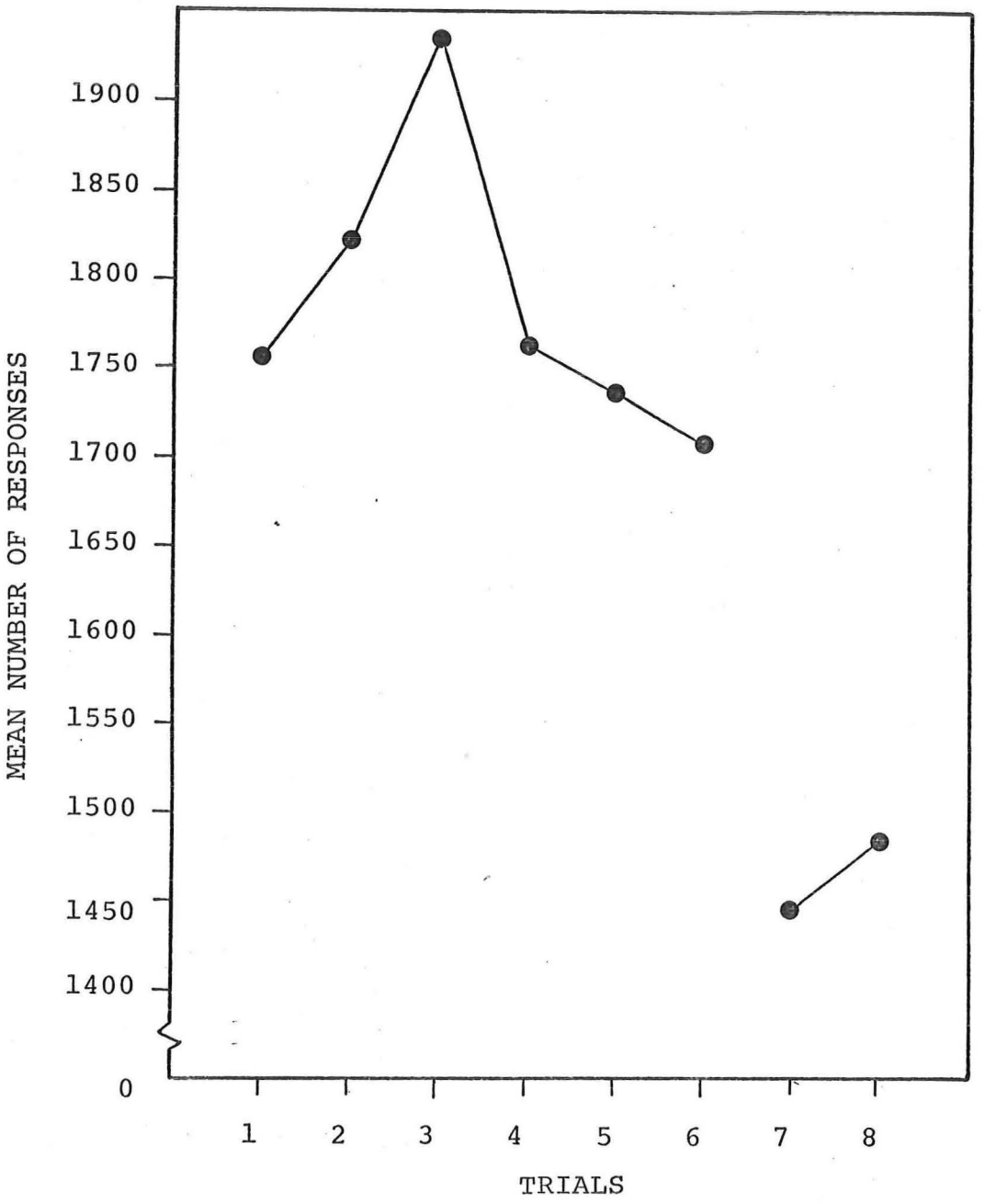

FIGURE 5: Mean total session responding (licks) before (trials 1-6) and after (trials 7-8) conditioning sessions.

On days 17 - 20 total number of responses and amount of water consumed during drug and non drug sessions were compared. Table 1 presents the mean number of responses (licks) and the respective standard deviation for saline and scopolamine sessions.

TABLE 1. Mean number of responses and their standard deviations for saline and scopolamine sessions on days 17 - 20.

	SALINE	SCOPOLAMINE
MEAN	1411.25	790.75
STANDARD DEVIATION	987.08	489.42

Comparison of mean responding in the drug and non drug conditions showed no statistically significant difference ($t(14) = 2.04$ N.S.), although there was a recognizable trend towards a decrease in responding after scopolamine administration. Table 2 presents mean water consumption (ml) and the respective standard deviation for saline and scopolamine sessions.

TABLE 2. Mean water consumption (ml) and standard deviations for saline and scopolamine sessions on days 17 - 20.

	SALINE	SCOPOLAMINE
MEAN	10.89	2.23
STANDARD DEVIATION	2.94	2.03

Comparison of mean water consumption showed a significant difference between drug and non drug conditions ($t(14) = 11.57$ $\rho<.001$) indicating that scopolamine produced a decrease in water consumption.

D. DISCUSSION

The results of the post test for conditioned effects demonstrate that scopolamine injected twenty minutes prior to conditioning sessions was ineffective in producing a suppression of responding in the presence of the flashing light CS. No differences were found between conditioned suppression ratios in the pre and post tests. The conditioning sessions also failed to produce any change in total water consumption. The conditioning regimen did produce a slight decrease in total responding, however as this

was not associated with a decrease in water consumption it is likely to have been a function of the subjects' increased mastery of the drinking task.

There are various possible explanations as to why the conditioning regimen did not produce conditioned suppression of responding. Firstly, the number of conditioning sessions may have been insufficient. Perhaps the four twenty minute pairings of the drug effect and the CS were not enought for the association to form. Secondly, the temporal incongruity of the CS and UCS. The onset and offset of a drug effect is considerably less distinct than that of other UCS's such as electric shock. The drug state is likely to be present before and after CS presentation, as well as during it. Thus the drug state is not associated solely with presentation of the CS. Also with respect to temporal factors, it may be the case that the major stimulus properties associated with the scopolamine drug state occur in the first twenty minutes after injection. This possibility is further investigated in Experiment III (Chapter VI). The use of an alternative CS might have produced stronger suppression. An auditory tone may have been more suitable than the flashing light CS used. Observation of the subjects while drinking indicated that they may have positioned themselves in such a way that the flashing light may not have been noticed. Their apparent concentration of visual attention on the

drinking nozzle may also have affected their perception of the CS. Conditioned suppression might have been more obvious had the drinking task involved bar pressing rather than nozzle licking. De Costa and Ayres (1971) have demonstrated that dipper licking is considerably more difficult to suppress than bar pressing. Finally, the effectiveness of a CS in suppressing responding is likely to be dependent on the effectiveness of the UCS in suppressing responding.

Although it has been demonstrated that scopolamine decreases liquid consumption (Stein, 1963; Prabham and Roth, 1968), other studies which have demonstrated conditioned suppression using a drug-state UCS have employed drugs, namely chlorpromazine and LSD-25 in doseages which by themselves will completely suppress responding (Cameron and Appel, 1972). The ability of the UCS to completely suppress responding might therefore be the necessary precondition for the CS to produce conditioned suppression. Thus on days 17 - 20 tests were carried out to assess the direct effects of the UCS, scopolamine, on responding and water consumption. The results demonstrated that scopolamine did not significantly disrupt responding, although there was a non statistically significant trend towards a decrease in responding. This failure of the UCS itself to suppress responding to any significant degree would explain why the CS did not suppress responding. The results of these trials

also demonstrated a significant decline in water consumption. This decline greatly exceeded the decline in responding. Mean responding declined by approximately 40% whereas mean water consumption declined by approximately 80%. In various individual cases subjects made up to 1000 responses without consuming any water. Thus the water deprived subjects were licking but not receiving water. Scopolamine appears to have disrupted the topography of licking in a way not evident to visual observation.

Although this study raises numerous interesting questions, the results remain largely uninterpretable with respect to the assessment of the aversive stimulus properties of scopolamine. It was decided that more conclusive results might be obtained using a different experimental paradigm.

CHAPTER V

EXPERIMENT II

A. INTRODUCTION

As a result of the uninterpretable nature of the findings of the conditioned suppression experiment it was decided that more conclusive results might be produced using one of the other designs for the assessment of drug stimulus properties. In this experiment the aversive stimulus properties of scopolamine were investigated using a conditioned location preference/avoidance paradigm. The research design used was similar to that employed by Reicher and Holman (1977) in their investigation of conditioned location preference/avoidance using amphetamine.

B. METHOD

(i) Subjects

Eight subjects were used. Their age range at time of testing was 166 - 174 days (mean = 168 days) and their weight range was 310 - 404 grams (mean = 355 grams). All subjects were provided with ad libitum food and water in the home cage.

(ii) Apparatus

The rats were individually trained and tested in a Lafayette Shuttlebox (Model A550), measuring 61.0 cm x 25.0 cm x 28.0 cm (L x W x H). The shuttlebox was divided lengthwise by a partition which contained an opening in its centre measuring 9 cm x 12 cm (W x H). The bottom of the opening was 1.5 cm above the grid floor. The opening could be closed by means of a guillotine door. The central partition and sides of the shuttlebox were constructed of stainless steel, with tinted glass plates at the front to allow observation of the subject. The floor consisted of 36 x 0.5 cm diameter stainless steel rods spaced 2 cm apart. The ceiling was translucent perspex. A Mazda K2K 200/250V 15W light bulb was positioned immediately above the ceiling of both chambers. Illumination levels in both chambers were 325 lx. On the end wall of each side of the shuttlebox was fixed the discriminative stimuli. On one side the wall was covered by 3 cm black and white vertical lines (see Figure 6) and on the other side 3 cm black and white horizontal lines (see Figure 7). A Lafayette white noise generator connected to a speaker positioned behind the shuttlebox provided background noise of 80 dB. Microswitches connected to the grid floor of the shuttlebox allowed Pye Hi-Log programming equipment to automatically record on counters the amount of time (0.5 second units) spent

in each side of the shuttlebox and the number of times the subject crossed from one side of the shuttlebox to the other.

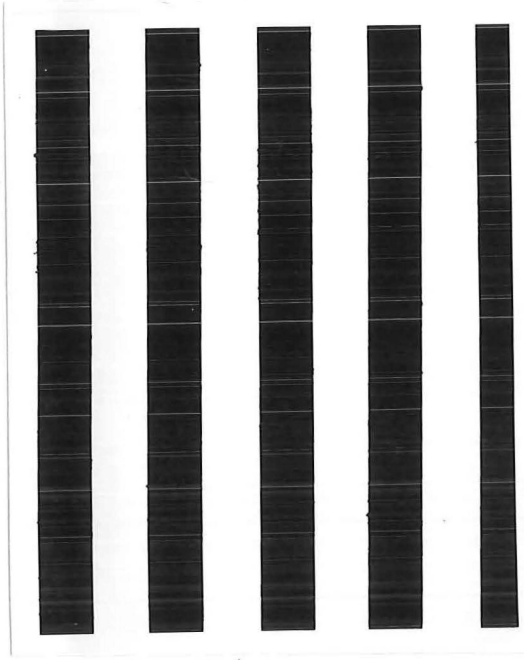

FIGURE 6: Vertical line discriminative stimulus

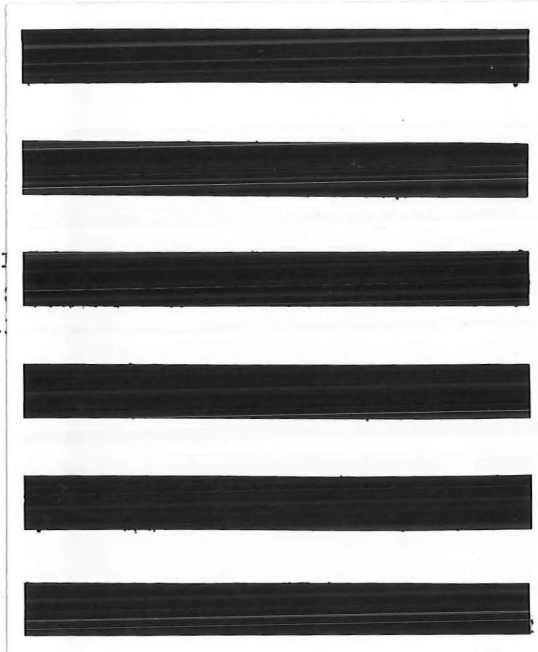

FIGURE 7: Hor
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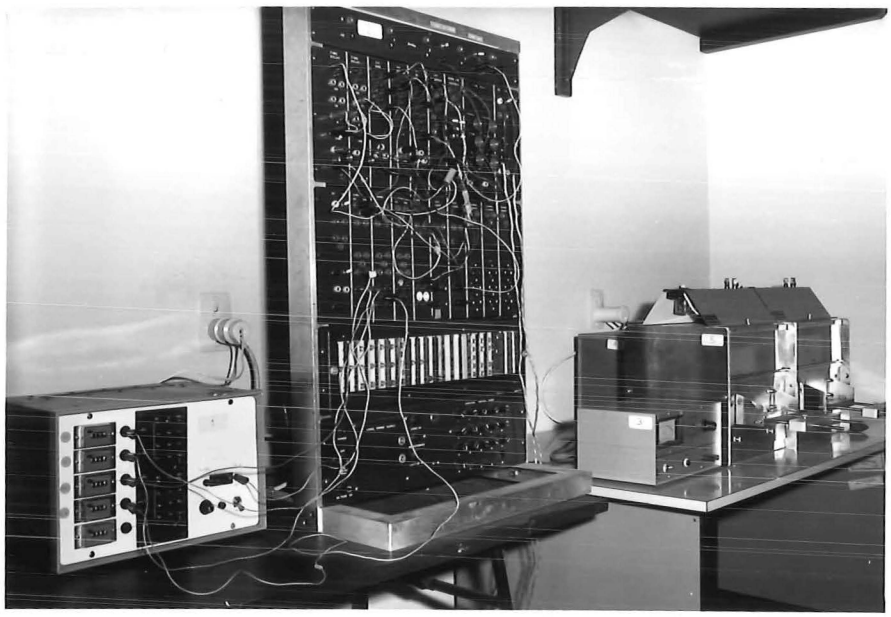

FIGURE 8: The experimental apparatus

1. Time unit counters
2. Pye Hi-Log programming equipment
3. Lafayette White Noise Generator (Model 15011)
4. White noise amplifier and speaker enclosure
5. Lafayette Shuttle Box (Model A550)

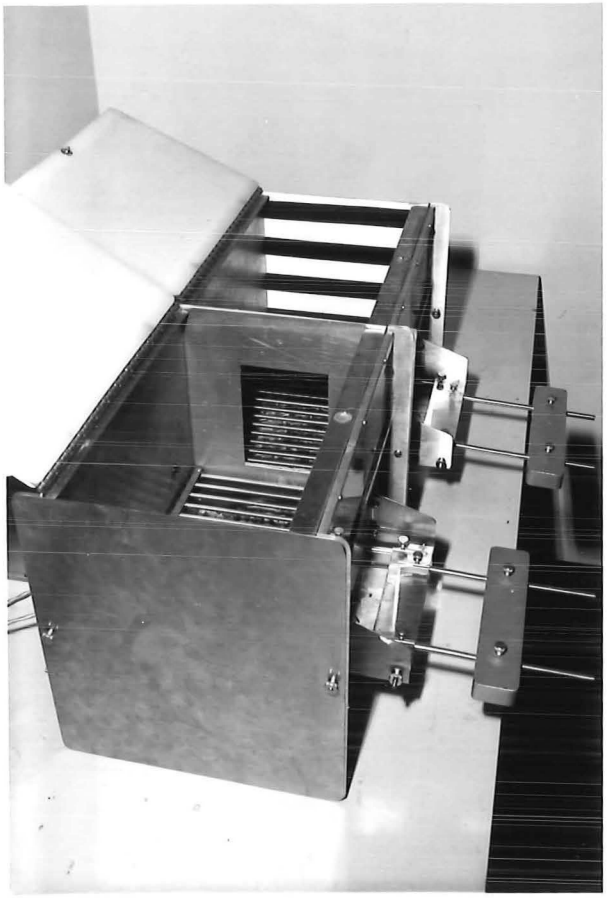

FIGURE 9: The shuttlebox

(iii) Procedure

Each subject received 22 consecutive daily sessions.

(a) Adaptation

On days 1 - 4 each subject received one session per day in the experimental chamber, without injection. Each subject was confined to the side with the vertical line stimulus (VS) or the side with the horizontal line stimulus (HS) on alternate days. Subjects were randomly assigned to two groups so that on one day, one group was confined to the VS side of the shuttlebox and the other group to the HS side, and then on the following day vice versa. Each subject thus received two sessions confined in the VS side and two sessions confined in the HS side (40 minutes total on each side).

(b) Test for Baseline Stimulus Preference

On days 5 - 6 the guillotine door was retracted and the subjects were individually placed in the apparatus with free access to both sides. On day 5 half of the subjects in each group were placed in the shuttlebox on the VS side and half on the HS side. On day 6 the sides of entry were reversed. After each session, the amount of time spent in each side of the shuttlebox and the number of times the subject crossed from one side to the other was recorded.

(c) Conditioning

On days 7 - 18 each subject was given an injection of either saline or scopolamine immediately before being confined to one half of the shuttlebox. Subjects were given scopolamine or saline on alternate days. After injection with scopolamine the rat was always placed on one side of the box, and placed on the other side when injected with saline. Subjects were thus confined to the VS or HS sides on alternate days. Subjects received six scopolamine sessions (120 minutes total) and six saline sessions (120 minutes total). Half of the subjects were placed in the VS side after scopolamine administration and half on the HS side. These two groups were divided so that half of each group was injected with scopolamine on any one day and the other half injected with saline. Thus there were four balanced subgroups each containing two subjects.

(d) Post Test for Conditioned Effects

On days 20 - 22 the procedure followed during the test for initial stimulus preference was duplicated with one modification. The side of entry to the shuttlebox was not alternated. One subject from each of the four subgroups was entered on the VS side on every day of the post test while the other was always entered on the HS side. No injections were given during this phase of the experiment.

C. RESULTS

The raw scores of location preference (i.e. time spent in each half of the apparatus) obtained in the post test (days 19 - 21) after conditioning sessions, were used to produce a single preference score for each animal for the side associated with saline injection. This score is the proportion produced by:

$$\frac{T_{SAL}}{T_{SAL} + T_{SCOP}}$$

T_{SAL} = time spent in side associated with saline injection

T_{SCOP} = time spent in side associated with scopolamine injection

Indifference, or equal preference for both sides, would produce a score of 0.50. The denominator of the above equation does not equal total time spent in the experimental apparatus as it does not include time spent crossing the midline between compartments of the shuttle-box. Scores obtained in the pre test trials (days 5 and 6),prior to conditioning sessions, were used in the same way to produce preference proportions for the side later to be associated with saline injection.

Figure 10 illustrates the mean daily preferences obtained in the pre test and post test.

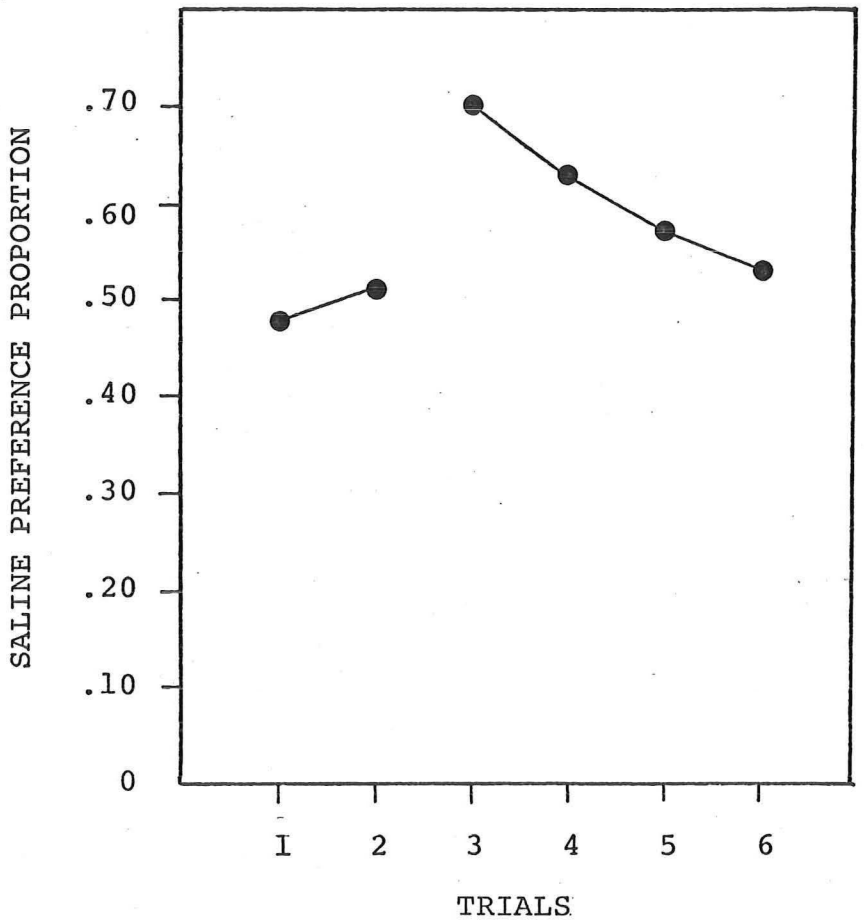

FIGURE 10: Mean daily preference score for the saline associated side of the apparatus before (trials 1-2) and after (trials 3-6) conditioning sessions.

The means for the first and second days of the pre test were 0.48 and 0.51,respectively. Comparison of these two scores produced no significant differences ($t(14) = 0.542$ N.S.). These results demonstrate that the subjects had no initial preferences for either side of the apparatus.

The means for the post test were 0.70, 0.63, 0.57 and 0.53, sequentially. A one way ANOVA with a planned a priori comparison of post test and pre test scores (Kirk, 1968, p.81) demonstrated a significant difference ($F(1,35) = 9.465$ $p<.01$). This indicates that the rats were spending a greater amount of time in the saline associated side during the post test after conditioning sessions. A second one way ANOVA showed this preference to decline significantly over days in the post test ($F(3,20) = 4.36$ $p<.05$). On day 22, the last day of the post test, the scores were indistinguishable from those obtained in the pre test ($t(14) = 0.35$ N.S.).

Figure 11 displays the mean daily frequency of midline crossings. The means for the first and second days of the pre test were 27.60 and 22.36 respectively. The means for the post test were 19.88, 19.88, 17.80 and 20.75, sequentially. The pre and post test scores were not significantly different ($F(6,35) = 1.69$, N.S.) although there was a trend towards decreased scores in the post test.

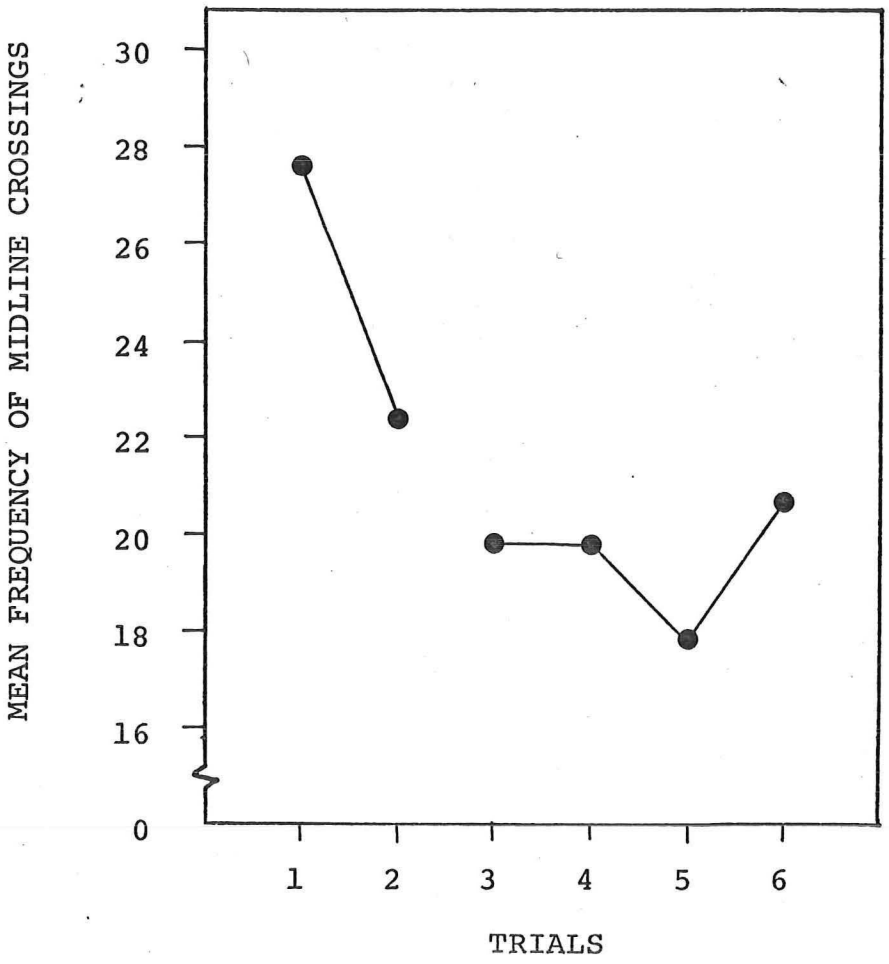

FIGURE 11: Mean daily frequencies of midline crossings before (trials 1-2) and after (trials 3-6) conditioning sessions.

D. DISCUSSION

The results of this experiment demonstrate that scopolamine injected immediately prior to conditioning sessions will produce a conditioned place avoidance. In the initial post test sessions the rats avoided the side of the apparatus previously associated with scopolamine administration, as indicated by the increased saline preference scores. This conditioned avoidance of the scopolamine associated side gradually extinguished over days in the post test until on the last day the side preference had returned to indifference.

The results establish that the stimulus properties of scopolamine when used in this particular conditioning regimen are aversive. This is consistent with the demonstrations of novelty avoidance attributed to an aversive action of scopolamine (Hughes and MacMahon, 1976, 1977; Hughes and Daley, 1977). In these latter studies previous exposure to the stimuli immediately followed scopolamine administration of a similar dose (1 mg/kg).

The trend towards decreased midline crossings is also consistent with similar trends towards a decrease in ambulation demonstrated in the novelty avoidance studies. It is generally believed that fearfulness and ambulation are negatively correlated (Archer, 1973).

Hughes (1978) has proposed that the suggestion that the highly novel experience of a drug state is in

itself aversive to a pharmacologically naive subject (Amit and Baum, 1970), could be relevant in explaining the role of scopolamine in the production of conditioned taste aversions (Berger, 1972) and novelty avoidance. The taste aversion studies involved only one drug conditioning session and the novelty avoidance studies involved two sessions. However results in the present study could not be explained in terms of aversiveness due to a novel experience as the subjects received a considerable number of drug trials. This would have allowed for habituation to any novel effects. It would therefore appear that scopolamine possesses aversive stimulus properties beyond those possibly resultant from the novelty of the experience of drug administration in pharmacologically naive subjects.

The results of this study provide evidence to support the hypothesis that the aversive properties of scopolamine could well be responsible for many of the results obtained in research utilising Carlton's Paradigm III (1968).

CHAPTER VI

EXPERIMENT III

A. INTRODUCTION

The possibility was raised in Experiment I that the production of a conditioned response, using scopolamine as the UCS, might be dependent upon the length of the pre injection interval i.e. the interval between injection and the start of the conditioning session. Evidence already existed which suggested that pre injection interval might be a variable of some consequence (Berger, 1972; Calhoun and Grant, 1974). In this experiment the paradigm used in Experiment II was duplicated with one modification. In the conditioning phase of the experiment a 20 minute pre injection interval was used i.e. subjects were injected 20 minutes prior to each conditioning session.

B. METHOD

(i) Subjects

Eight subjects were used. Their age at time of testing was 140 days. Their weight range was 325 - 440 grams (mean = 371 grams). All subjects were provided

with ad libitum food and water in the home cage.

(ii) Apparatus

As for Experiment II.

(iii) Procedure

The procedure followed in Experiment II was duplicated with one modification. During the conditioning phase of the experiment the subjects were injected and then returned to the home cage for twenty minutes before being confined to one or the other sides of the shuttlebox.

C. RESULTS

The raw scores of location preference in the pre and post tests were used as in Experiment II to produce a preference proportion score for the saline associated side. Figure 12 illustrates the mean daily preference scores obtained in the pre test (days 5 and 6) and the post test (days 19 - 22).

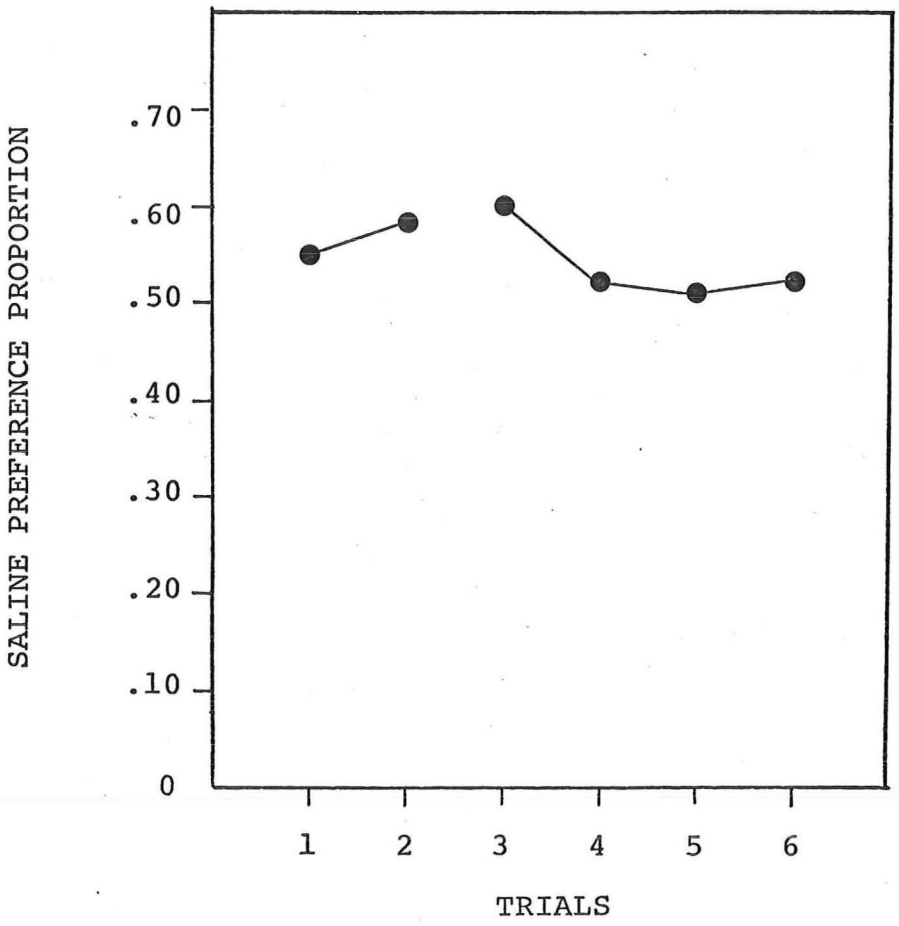

FIGURE 12: Mean daily preference score for the saline associated side of the apparatus before (trials 1-2) and after (trials 3-6) conditioning sessions.

The means for the first and second days of the pre test were 0.55 and 0.58 respectively. This indicates a slight initial preference for the saline associated side. The means for the post test were 0.60, 0.52, 0.51 and 0.52, sequentially. Comparison of pre and post test scores of location preference, using a one way ANOVA, showed no significant differences ($F(6,35) = 1.253$ N.S.) indicating that the conditioning sessions had not affected the animals' preferences for either side of the apparatus.

Figure 13 displays the mean daily midline crossing scores. Pre test means were 28.2 and 26.4 respectively. Post test means were 28.3, 26.6, 29.1 and 24.5, sequentially. No significant differences were found between the pre and post test scores ($F(6,35) = 1.609$ N.S.).

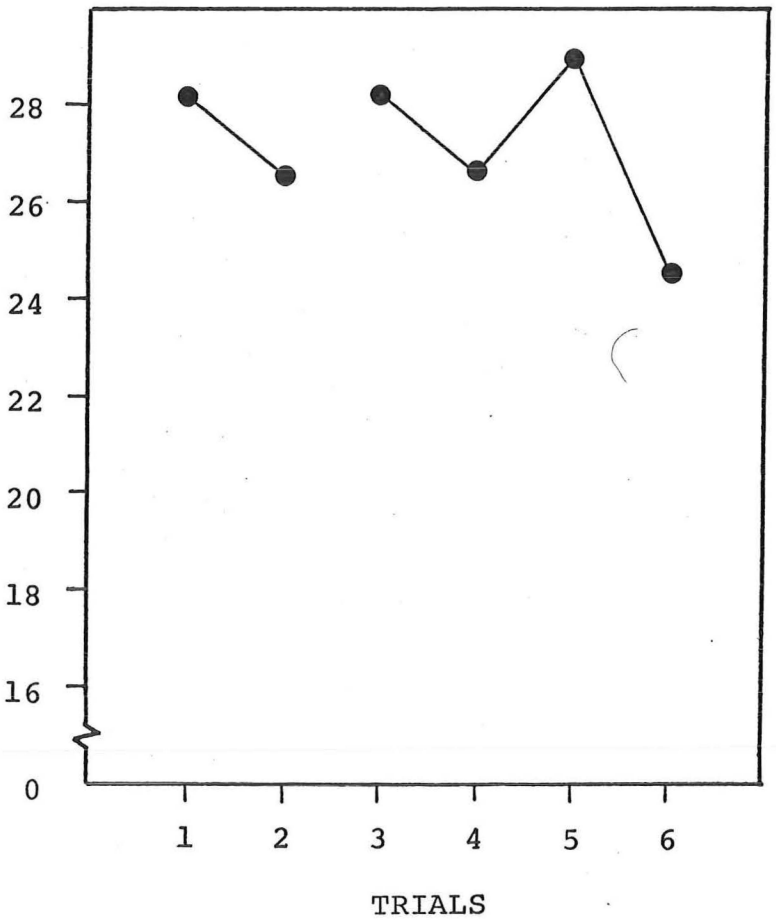

FIGURE 13: Mean daily frequencies of midline crossings before (trials 1-2) and after (trials 3-6) conditioning sessions.

D. DISCUSSION

The results of this experiment demonstrate that scopolamine injected twenty minutes prior to conditioning sessions is ineffective in producing a conditioned place avoidance. Side preferences in the post test after conditioning sessions were not significantly different from those obtained in the pre test. These results are consistent with midline crossing scores which did not differ between pre and post tests. It would appear that the stimulus properties of scopolamine injected twenty minutes prior to conditioning sessions are not aversive in the conditioning of this particular response.

Taking into account the results obtained in Experiment II it would seem that the stimuli necessary for the production of a conditioned place avoidance occur in the first twenty mintues after drug administration. It is proposed that it is the onset of the scopolamine drug state that is the most aversive element, rather than the drug state itself. The 20 minute delay is generally seen as being sufficient to allow the drug to take full effect. This belief is based on the report of Buresova et al (1964) that atropine administered intraperitoneally in 5 mg/kg to rats had its maximal effect on the EEG twenty minutes after injection.

The pre injection time of scopolamine has previously been shown to be an important variable in step down

passive avoidance latency in mice (Calhoun and Grant, 1974). Subjects which received the drug immediately before the test performed normally while performance was impaired for subjects injected 5 or 10 minutes before the test. Although it is likely that scopolamine functions in the latter study in a very different way to that proposed as an explanation of the results in the present studies, it nevertheless shows pre injection interval to be a variable of possible consequence.

The results of the present study are consistent with those of Berger (1972) who demonstrated that conditioned taste aversion was not obtained if scopolamine (1 mg/kg) was injected 30 minutes prior to milk drinking. Berger's study did not include an immediate pre injection condition. As previously noted Berger explained this in terms of Pavlovian conditioning procedures requiring that the CS precede the UCS. However as conditioned taste aversions have been produced with pre CS injections of drugs such as amphetamine (Reicher and Holman, 1977) it is possible that conditioned taste aversions may be produced by immediate pre injection of scopolamine.

It could be said that the proposed amnesic properties of scopolamine (e.g. Carlton, 1963) could account for the results of this study, i.e. after 20 minutes consolidation processes are impaired so that memory for an association between the CS and the drug's aversiveness is disrupted. However if this was the case it would seem

probable that a preference for the scopolamine associated side would have been demonstrated. Memory disruption would be associated with habituation deficits and thus the scopolamine associated side would be a more novel stimulus than the saline associated side.

CHAPTER VII

CONCLUSION

It has been the aim of the experiments in this study to investigate the hypothesis that scopolamine, administered to rats, has aversive stimulus properties that may well account for or have influenced many of the reported effects previously attributed to the central effects of the drug (Hughes and MacMahon, 1976, 1977). The study has demonstrated that immediate pre injections of 1 mg/kg scopolamine during conditioning will produce a conditioned place aversion to the side of a shuttlebox associated with scopolamine administration. This result clearly demonstrates that the stimulus properties of the drug when administered in this paradigm are aversive. The study has also demonstrated that if scopolamine injections are administered 20 minutes prior to the conditioning sessions a conditioned place aversion will not be produced. It would therefore appear that the stimulus elements necessary for the production of a conditioned place aversion occur in the first 20 minutes after injection. It is proposed that it is the onset of the drug effect rather than the drug effect per se that possesses the major aversive stimulus properties.

The first experiment in the study, that using the

conditioned suppression paradigm, also involved a 20 minute pre injection interval, and also failed to produce a conditioned effect. However as previously noted, this result is not necessarily solely the result of the pre injection interval. Many factors may have been in operation, perhaps one of the most important being the inability of the drug itself to suppress responding. An interesting by-product of this experiment was the observation that scopolamine appeared to disrupt the topography of the licking response. Although responding was not significantly altered, water consumption was decreased considerably.

When considering the results of Experiment II, it is important to recognise that the stimulus properties of scopolamine have been demonstrated to be aversive only in the context of the measure under consideration i.e. location preference. The aversive properties of the drug will not necessarily be reflected in the conditioning of other responses. Reicher and Holman (1977) have clearly demonstrated the importance of this consideration in their observation that amphetamine can simultaneously produce a positively reinforced preference for the side associated with amphetamine and an aversion to the flavoured solution associated with the drug. Although scopolamine administration has demonstrated a little more consistency, with the drug producing both location and taste aversions, it nevertheless remains

important not to generalise the stimulus properties to other contexts without empirical evidence.

The failure of the 20 minute pre injection condition to produce a conditioned location aversion should also be considered with some caution. It may well be the case that had the conditioning regimen been of shorter duration, an aversion may have been produced due to the aversive properties of the novel drug state to the naive animals (Amit and Baum, 1970). The length of the conditioning phase of the experiment precluded this as a feasible explanation of the results in this study. In this context, a future direction for research could involve analysis of the length of conditioning regimens in order to discriminate between stimulus properties associated with a novel drug state and stimulus properties unique to the particular drug under consideration.

The results of this study are only preliminary in the assessment of the stimulus properties of scopolamine. Many directions exist for future research. Firstly, further research is needed to investigate the drug's effectiveness as a UCS in the conditioning of other responses. As previously suggested it might well be the case that immediate pre injection of scopolamine will produce a conditioned taste aversion. It would also be of interest to test the drug's ability to suppress bar pressing for water reinforcement and subsequently its ability to produce a conditioned suppression of bar pressing.

Bar pressing is reported to be more easily disrupted than licking (De Costa and Ayres, 1971). Investigation of the effectiveness of scopolamine in an instrumental conditioning paradigm as previously described would also be valuable.

A thorough assessment of the stimulus properties of scopolamine must also involve the examination of various dosages. Hughes, Blampied and Stewart (1975) in a study investigating scopolamine induced changes in activity and reactions to novelty, demonstrated complex, non linear relationships between dose strength, within session response decrement and the three separate behaviour measures recorded (cells entered, rearing, and reactions to novelty).

The study demonstrated that maximum declines in all cells entered and rearing occurred with the lowest dosages (0.1, 0.25 mg/kg). The greatest tendencies for novelty avoidance also occurred with the lowest doses. A U shaped dose-response curve was reported for ambulation. Such findings clearly indicate that the effects of scopolamine cannot be accounted for by a single process explanation. They also demonstrate that the stimulus properties of various dose strengths of scopolamine may vary in a non linear fashion.

To assess the relative contributions of the peripheral and central stimulus properties of scopolamine in the conditioning of a response, a condition could

be employed whereby subjects received injections of scopolamine methyl nitrate rather than scopolamine. As noted previously, quaternary anticholinergics do not readily enter the central nervous system and thus their effects are due largely to their influence on the peripheral nervous system. Quaternary anticholinergics have previously been demonstrated to be an effective UCS in the conditioning of responses such as taste aversions (Berger, 1972) and paradoxical salivation and classical mydriasis (Koral et al, 1966). With respect to the present study, duplication of Experiment II using an equivalent dose of scopolamine methyl nitrate instead of scopolamine hydrobromide would provide evidence as to the relative contributions of central and peripheral stimulus properties in the conditioning of location aversions.

The demonstration that scopolamine, in certain contexts, possesses aversive stimulus properties capable of altering subsequent responding, clearly shows that this property must be controlled for or at least taken into account in other pharmacological research employing the drug. No longer can the drug's behavioural effects be attributed solely to its central effects. Before any conclusions can be drawn concerning the role of central cholinergic systems in the control of behaviour, evidence based on research using scopolamine must be shown not to be simply the result of the stimulus properties of

he drug. As has already been argued, many studies involving the use of scopolamine in the determination of the behavioural correlates of the cholinergic system (such as habituation to novelty) are more parsimoniously interpreted as being the result of aversive stimulus properties. It is not the intention of the author to propose that all the reported behavioural effects of scopolamine are the result of the drug's aversive stimulus properties, but rather that the stimulus properties may well have been influential in attaining the results, and that this factor must be controlled for. The same cautionary rule concerning control for stimulus properties should also be applied to studies involving any other drugs used as research tools to elucidate the functioning of the central nervous system.

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